Electrotransfer of Proteins

(Electroblotting)

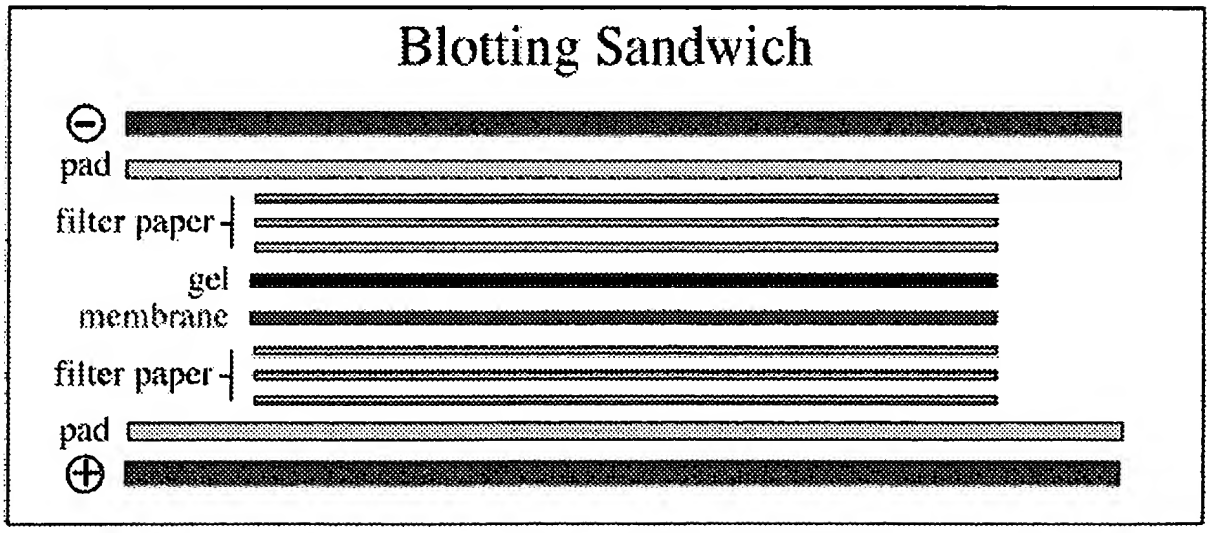
Solutions:

Towbin Transfer Buffer:	25 mM Tris	per liter: 3.03 g Tris base
	192 mM Glycine	14.41 g Glycine
	0.1 % SDS	1.00 g SDS
	20 % MeOH	200 mls MeOH
	(will be approx pH 8.3)	

- higher SDS concentrations facillitate extraction of proteins from gel but decrease binding of proteins to membrane
- higher MeOH concentrations increase binding of proteins to the membrane but decrease efficiency of transfer from gel (in practice use 20-25%)

Procedure

1. equilibrate membrane
   - nitrocellulose: wet in water and then equilibrate 15 minutes in transfer buffer
   - PVDF: wet in 100% MeOH briefly and then equilibrate 15 minutes in transfer buffer
2. equilibrate gel in transfer buffer for 15 minutes, especially if transfer buffer contains MeOH which will cause the polyacrylamide gel to shrink slightly
3. assemble transfer "sandwich"



- avoid bubbles which will block transfer of proteins
- pre-wet layers in transfer buffer
- assemble by laying layers down beginning near the center and lowering the edges
- roll pipette or test tube across sandwich to expel any air bubbles
- trim layers to size of gel to prevent "short-circuiting" which will reduce transfer efficiency
- use enough Whatmann 3MM filter paper to make sandwich tight in cassette

4. lower cassette into electrophoresis tank (wet electroblotting system) or between electrodes (semi-dry system) with membrane towards the anode (+) electrode
5. electrophoretic transfer times:
    1. tank system:
        - 100 V for one hour
        - 15 V overnight
        - 400 mA (constant current) for 3-5 hours
        - note: larger proteins will require more time to transfer and/or higher voltages
    2. semi-dry system:
        - 1 mA / cm2 gel area for 1.5 hours
6. membranes can be stored dry -- re-wet before processing (nitrocellulose in aqueous, PVDF in MeOH then aqueous)

Stain Membrane for Total Protein (Optional)

Useful for marking/aligning MW standards and lanes (mark with pencil or papermate ink) or for verifying effective transfer

Procedure:

1. Stain membrane for 1 minute in Ponceau S (0.3% PonceauS in 5% TCA)
2. Destain with several rinses of water

Troubleshooting Tips:

1. spots on membrane where no transfer occurs indicates air bubbles in the sandwich -- roll tube across sandwich to expel air bubbles
2. distortion of bands:
    - gel swelling during transfer due to excessive increase in temperature -- transfer at lower voltage or in the cold room
    - gel sandwich too loose within the transfer cassette -- use additional layers of Whatmann filter paper
    - gel shrinkage in MeOH -- equilibrate gel in transfer solution before assembling sandwich
3. poor protein transfer
    - lower acrylamide concentrations allow better transfer of higher molecular weight proteins
    - add higher percentage of SDS to transfer buffer

- use lower pore size membrane (i.e. 0.1 to 0.2 um pores)
- add additional membrane on cathode (-) side of gel to capture any proteins with abnormal isoelectic points

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compiled by Chad Rappleye

Aroian Lab Protocols

L Number	Hits	Search Text	DB	Time stamp
1	4	"6503747"	USPAT; US-PGPUB; DERWENT	2004/02/10 09:43
2	2132	towbin	USPAT; US-PGPUB; DERWENT	2004/02/10 09:08
3	0	towbin near5 edta	USPAT; US-PGPUB; DERWENT	2004/02/10 09:08
4	87724	towbin dame edta	USPAT; US-PGPUB; DERWENT	2004/02/10 09:08
5	143	towbin same edta	USPAT; US-PGPUB; DERWENT	2004/02/10 09:08
6	477	FTA	USPAT; US-PGPUB; DERWENT	2004/02/10 09:43
8	70	FTA same (cell$5 or buccal)	USPAT; US-PGPUB; DERWENT	2004/02/10 09:43

US-PAT-NO: 6322792

DOCUMENT-IDENTIFIER: US 6322792 B1

TITLE: Rhadino virus LANA acts in trans on a unit of rhadino virus DNA to mediate efficient episome persistance

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Detailed Description Text - DETX (66):

If LANA mediates the efficient segregation of KSHV episomes to progeny cells, then LANA expressing cells that are Z6 transfected and G418 resistant should contain extrachromosomal Z6 DNA. Extrachromosomal DNA should rarely or never be found in G418-resistant cells that are Z6 transfected and LANA negative or Z8 transfected and LANA positive. Gardella gel analysis followed by Southern (DNA) blotting was performed to assay whether Z6 DNA is an episome in BJAB/LANA and BJAB/F-LANA cells. In Gardella gels, live cells are lysed in situ in the gel loading wells at the start of the gel run. Episomal DNA (as large as 200 kb) migrates into the gel while chromosomal DNA is unable to migrate into the gel (Gardella gels were prepared as described in T. Gardella, P. Medveczky, T. Sairenji, C. Mulder, J. of Virol., 50, 248 (1984)). As expected, BCBL-1 (FIG. 4A, lane 1; FIG. 4B, lane 4) and KSHV infected BC-1 PEL cells (E. Cesarman, et al., Blood, 86, 2708 (1995)) (FIG. 4B, lane 2) had episomal KSHV DNA, whereas KSHV negative Raji (FIG. 4A, lane 2; FIG. 4B, lane 1) and BJAB (FIG. 4B, lane 3) cells lacked KSHV episomes. BJAB/LANA cells (FIG. 4A, lanes 3-7) or BJAB/F-LANA cells (FIG. 4A, lanes 8-12) that had grown out after transfection with Z6 DNA and G418 selection also had extrachromosomal DNA. In contrast, BJAB/F-LANA cells that had grown out after transfection with Z8 DNA and G418 selection did not have extrachromosomal Z8 DNA (FIG. 4B, lanes 5-12). Also, LANA negative BJAB cells that had grown out as G418 resistant after transfection with Z6 or Z8 did not have episomal DNA. These latter cells

had Z6 or Z8 DNA by polymerase chain reaction (PCR), and Z6 or Z8 DNA was
sometimes detected at the loading wells on long exposures of Southern blots of
Gardella gels, which is consistent with the presence of integrated DNA in these
cells. These experiments demonstrate that LANA acts in trans on a cis-acting
element present in Z6 to efficiently mediate Z6 episome persistence in cells.

US-PAT-NO: 5670352

DOCUMENT-IDENTIFIER: US 5670352 A

TITLE: Stable growth transformation of human T-lymphocytes by Herpesvirus saimiri (H. saimiri) subgroup C

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Detailed Description Text - DETX (11):
Total cellular DNA was isolated and analyzed by Southern blot hybridization to the Acc I fragment specific for strains of group C (FIG. 1) according to standard protocols (Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory, Manual (Cold Spring Harbor Lab., Cold Spring Harbor, N.Y.). To determine whether the viral genome was integrated into cellular DNA or persisted as an episome, 1.times.10.sup.6 cells were lysed on top of a 1% agarose gel by the procedure of Gardella et al. (Gardella, T., et al. (1984) J. Virol. 50, 248-254), and fractionated DNA was transferred to nitrocellulose filters. Hybridization was performed with a Kpn I fragment conserved in all virus strains (FIG. 1).

US-PAT-NO: 6503747

DOCUMENT-IDENTIFIER: US 6503747 B2

TITLE: Serotype-specific probes for Listeria monocytogenes

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Detailed Description Text - DETX (82):
grown overnight at 22.degree. C. Bacterial colonies were transferred onto nitrocellulose membranes (Micron Separations Inc.) presoaked in Towbin transfer buffer (Ausbel et al. Current Protocols in Molecular Biology. Green Publishing Associates and John Wiley and Sons, Inc. New York, N.Y.). The nitrocellulose membranes were air dried for 15 min. and processed according to standard immunoblot procedures (Kathariou et al. 1994. Appl. Environ. Microbiol. 60:3548-3552). MAbs c74.22 and c74.33 were used as ascites at a 1:400 dilution. MAb binding was demonstrated using goat antimouse-horseradish peroxidase conjugate (1:1000 dilution; Fisher).